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Urban Feral Pigeons (*Columba livia*) as a Source for Air- and Waterborne Contamination with *Enterocytozoon bieneusi* Spores▽

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This study demonstrated that a person with 30 min of occupational or nonoccupational exposure to urban feral pigeons, such as exposure through the cleaning of surfaces contaminated with pigeon excrement, could inhale approximately 3.5×10^3 *Enterocytozoon bieneusi* spores and that 1.3×10^3 spores could be inhaled by a nearby person.

Microsporidia are on the Contaminant Candidate List of the U.S. Environmental Protection Agency due to their unknown transmission routes, the challenging spore identification process (14), and the complex treatment of infections with these organisms (5). The zoonotic transmission of *Enterocytozoon bieneusi* remains a possibility as this species infects a wide range of mammals (4, 13) and birds (9, 10, 12, 13, 15). Most metropolitan cities are inhabited by large populations of feral pigeons, creating the opportunity for frequent contact with humans (8). Thus, there is a potential risk of spore transmission from feral pigeons to people through occupational exposure or casual interactions. The purpose of the present study was to determine if long-term feral pigeon congregation sites present any public health or occupational risks for the transmission of *E. bieneusi* spores to human populations.

The study site was a surface depression on the flat, tar paper-covered roof (12 by 48 m, i.e., 576 m^2) of a three-floor townhouse in Baltimore, MD (76°35′21.42″W, 39°17′52.71″N). The rooftop depression was a visible, permanent, and undisturbed gathering site for large numbers of feral pigeons (*Columba livia*). Feral pigeons used this site throughout the year for bathing, defecating, sheltering, and cooling down, and the pigeon population fluctuated between 10 and approximately 550 daily. After several years, such conditions resulted in a thick (approximately 10-cm) deposition of pigeon excreta, i.e., guano. Five 1-liter water samples with sediments and two samples (approximately 0.5 kg each) of guano were collected at approximately 3-week intervals. Air was sampled during dry weather while the rooftop depression was swept with a heavy-duty contractor broom to simulate conditions of heavy disturbance. Air was sampled continuously during the 30-min sweeping period by using two personal air samplers and an area sampler, i.e., Biosampler (3, 11).

Water and guano samples were gravity sedimented overnight at 4°C (2), 50 ml of the top layer was collected into a sterile plastic tube and centrifuged (5,000 × *g* for 10 min), the supernatant was discharged, and the pellet was processed by sugar-phenol flotation (2). The phosphate-buffered saline (PBS) from the Biosampler was centrifuged (5,000 × *g* for 10 min), the supernatant was discharged, and the pellet was resuspended in 100 μl of sterile PBS. Air filters from the personal sampling devices were dissolved in 1.5 ml of sterile PBS in a water bath at 45°C. The suspension was centrifuged at 45°C (5,000 × *g* for 10 min), the supernatant was discharged, and the pellet was resuspended in 100 μl of sterile PBS. Duplicate direct wet smears prepared from all samples were air dried, fixed with methanol, and stained with Chromotrope-2R and calcofluor white M2R (2). The remaining samples were coded, and multiplex fluorescence in situ hybridization assays for *E. intestinalis*, *E. hellem*, *E. cuniculi*, and *E. bieneusi* in 1.5-ml microcentrifuge tubes were carried out as described previously (6, 7, 16). For confirmation, one combined sample of water and two air samples were assayed by PCR (6).

The multiplex fluorescence in situ hybridization assays identified potentially viable *E. bieneusi* spores in all water, guano, and air samples (Table 1). The overall concentrations of *E. bieneusi* spores in water and guano samples were 3.8×10^4/liter and 3.6×10^3/g, respectively (Table 1). The total numbers of *E. bieneusi* spores recovered by the two personal air samplers were 1.1×10^3 and 1.0×10^3, resulting in concentrations of airborne spores of 1.8×10^4 and $1.7 \times 10^4/m^3$, respectively (Table 1). The total number of *E. bieneusi* spores recovered from the Biosampler was 3.2×10^3, resulting in an airborne-spore concentration of $0.9 \times 10^4/m^3$ (Table 1). PCR amplifi-

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TABLE 1. Detection of *E. bieneusi* spores in water, pigeon guano, and disturbed-condition air samples collected at a long-term feral urban pigeon (*C. livia*) congregation site

Sample type (*n*)	Concn(s)[a] of microsporidian spores	% of potentially viable spores
Water with sediments (5)	4.7×10^4, 3.1×10^4, 3.9×10^4, 3.5×10^4, 3.8×10^4	65
Pigeon guano (2)	3.5×10^3, 3.7×10^3	85
Air, personal samplers (2)	1.8×10^4, 1.7×10^4	30
Air, Biosampler (1)	0.9×10^4	25

[a] Concentrations are expressed as the number of spores per liter (water), gram (guano), or cubic meter (air).

cation confirmed the presence of *E. bieneusi* DNA among DNA extracted from spores in water and air samples.

The present study demonstrated that (i) *E. bieneusi* was the only microsporidian parasite recovered from the urban feral pigeon congregation site that is virulent in humans; (ii) *E. bieneusi* spores can be aerosolized from disturbed excrements of pigeons and may potentially be inhaled by humans as airborne particles; and (iii) air, water, and pigeon guano may contain potentially viable *E. bieneusi* spores and, thus, can serve as a source of air- and waterborne contamination.

Given a breathing volume of 9,600 liters/8 h for a moderately active person (1) and the concentration of airborne *E. bieneusi* spores determined by personal air samplers (Table 1), the present study demonstrated that a person with 30 min of occupational exposure to pigeons, e.g., through cleaning surfaces contaminated with their excrements, could inhale approximately 1.05×10^4 spores. Given the fact that approximately 30% of airborne spores were potentially viable, the number of inhaled viable spores may reach approximately 3.5×10^3. The data on the concentration of *E. bieneusi* spores determined by the Biosampler indicate that a person standing for 30 min within an area that contained pigeon excrements while disturbance was conducted could inhale a total of 5.4×10^3 spores, of which approximately 1.3×10^3 would be potentially viable.

The total rainfall for the study area during the sampling period (28 June 2006 to 4 October 2006) was 26.6 cm (http://www.weather.gov/climate/index.php?wfo=lwx). Given the size of the rooftop drainage of 12 by 48 m (576 m^2) and the maximum volume of the rooftop depression of approximately 350 liters, the accumulation of pigeon guano at this site was flushed over 4.5×10^2 times with rainwater during the sampling period. As the average concentration of waterborne *E. bieneusi* spores at this site was 3.8×10^4/liter, the rainwater runoff from this pigeon congregation site delivered an enormous number of *E. bieneusi* spores to the nearby urban storm water runoff system.

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